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## Dexamethasone decreases neurological sequelae and caspase activity

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**Abstract Objective:** To evaluate the use of dexamethasone in a model of meningitis-induced brain injury. Changes in neurobehavioral performance were the primary outcome variables. Changes in caspase activation and markers of neuronal injury were the secondary outcome variables. **Design:** Randomized, prospective animal study. **Setting:** University research laboratory. **Subjects:** Male Wistar rats. **Interventions:** Animals underwent a basilar cistern injection of either placebo or a suspension of Group B Streptococcus. Sixteen hours after inoculation, animals were randomized and received either dexamethasone or placebo in addition to antibiotics. Neurobehavioral performance and biological markers of brain injury were assessed at 3 days and 9 days after randomization. In a second experiment, caspase 1 and 3 were evaluated at 6 h, 24 h, and 72 h after dexamethasone administration. **Measurements and main results:** Neurobehavioral performance at 3 days and 9 days was significantly im-

proved in the dexamethasone group. Serum C-tau and cerebral edema were decreased after 3 days of dexamethasone treatment. Dexamethasone decreased Caspase 3 activation in meningitic animals.

**Conclusion:** These findings demonstrate that dexamethasone decreases acute brain injury in a rat model of bacterial meningitis as measured by preservation of neurobehavioral performance.

**Keywords** Meningitis · Brain injury · Caspase · Neurobehavioral performance

### Introduction

Meningitis generates a complex brain injury causing focal, neuropsychological, and intellectual impairments, with the participation inflammatory and apoptotic pathways independent of the causative organism [1, 2, 3]. Brain injury as a result of meningitis, similar to that of other causes of brain injury, is a consequence of the combination of primary and secondary injury [4]. Sec-

ondary injury, the target of most therapeutic interventions, is a sequence of biochemical and physiological processes that exacerbate the initial insult [4]. The inflammatory response is a frequently targeted component of secondary brain injury, the apoptotic pathway a novel one. Steroids, particularly dexamethasone, have been proposed as a potential agents to decrease inflammation. However, throughout the brain injury-recovery continuum, the effects of dexamethasone are diverse and conflicting due to induc-

tion of apoptosis, interference with trophic support, and learning process [6, 7, 8, 9, 10]. Despite its theoretical promise, the effect of dexamethasone on changing the outcome in clinical studies of meningitis has been less straightforward. While steroids treatment has received a cautious endorsement by the AAP Committee on Infectious Disease (Redbook), a recent study has created a renewed interest in the possible salutary effects in meningitis [11].

In previous studies we demonstrated that hypothermia can reduce the inflammatory response and biomarkers of brain injury but fails to improve neurobehavioral performance [12]. Ultimately, no improvement in any collection of mediators and markers has any significance if that improvement is not associated with an improvement in the cognitive and motor injury to be endured by the patient. In this study, we sought to investigate whether dexamethasone would improve neurobehavioral performance in a rat model of bacterial meningitis, and compare these results to specific biomarkers of brain injury.

## Material and methods

A randomized, prospective animal study was conducted. Mature male Wistar rats received either sterile saline (Sham) or an equivalent volume of a Group B Streptococcus-type III (GBS) suspension ( $5 \times 10^9$  cfu/ml) in the cisterna magna while under anesthesia. Animals received saline 10 ml and buprenorphine (0.15 mg) SQ before being returned to their cages.

Sixteen hours post-inoculation, brain injury severity was assessed by two observers using a clinical severity score (css) as previously described [12]. Animals were stratified and paired by their css, and randomized to receive either dexamethasone (Dex) (0.6 mg/kg-day up to 4 days SQ), or placebo; all animals received Ceftriaxone (100 mg/kg-day up to 9 days SQ). Infected animals were enrolled until there was a minimum of seven survivors in each group: 1) Placebo (bacteria and placebo); 2) Dex (bacteria and dexamethasone); and 3) Sham (no bacteria and placebo). Animals were killed with an overdose of pentobarbital.

Protocol 1: neurobehavioral performance and biological markers of neuronal injury

Rats were trained on neurobehavioral test 2 weeks preceding inoculation. Before killing, animals were weighed and underwent neurobehavioral testing. An intracardiac puncture for blood collection was performed prior to sacrifice.

### Neurobehavioral tests

Rotorod Test (motor coordination), the apparatus, training and testing technique utilized were as we have previously described [13]. Water maze (learning acquisition and memory retention), equipment, training and testing were performed as previously described [13]. Before killing, 3 days and 9 days post randomization, animals were given four trials of 5 min each to locate the submerged platform. The dependent measure was the number of trials required to meet a time criterion (25 s = mean+2SD from their final pretreatment test).

Quantification of serum C-tau proteins was performed by ELISA as previously described [13]. Cerebral edema was determined on animals killed after 3 days as previously described [14].

### Protocol 2

#### Caspase activation

Infected animals were subdivided into killing at 6 h, 24 h, and 72 h after dexamethasone administration. Sham animals were killed at 24 h. Animals underwent terminal perfusion and the cranial content were flash frozen. Assays of caspase activity were done in cerebellum.

Activity of Caspase 1 and Caspase 3 were measured by the cleavage of the fluorogenic tetrapeptide amino-4-methylcoumarine conjugate (YVAD-AFC for Caspase 1 and DEVD-AFC for Caspase 3) in cerebellum cytosol extracts [15].

#### Analysis

All data are presented as mean±standard error for each experiment. Differences among groups were determined by unpaired *t*-test or ANOVA followed by Bonferroni correction for multiple comparisons. Regression analysis was utilized when a relationship between two continuous variables was explored. Results were considered significant at  $P < .05$ .

## Results

### Protocol 1

The css were not different between infected groups: 3 days: placebo ( $n=8$ ) css  $1.3 \pm 0.3$ ; Dex ( $n=7$ ) css  $1.25 \pm 0.3$ ; Sham ( $n=5$ )  $0.2 \pm 0.2$ ; and 9 days: placebo ( $n=7$ ) css  $1.4 \pm 0.4$ , Dex ( $n=7$ ) css  $1.5 \pm 0.3$ ; and Sham ( $n=2$ ) css 0.

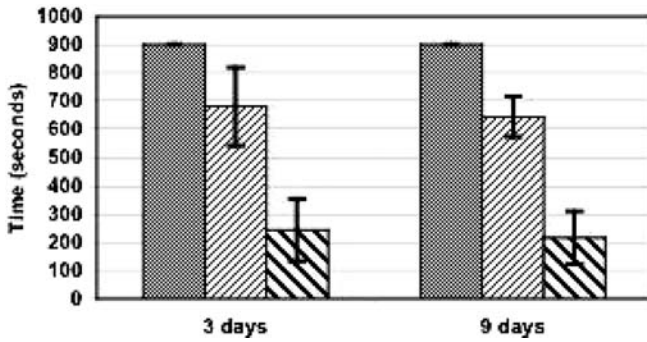
#### Neurobehavioral performance

Dex groups had a significantly improved performance over placebo groups: 3 days (placebo  $244 \pm 110$ , Dex  $680 \pm 139$   $P < .05$ , Sham  $900 \pm 0$  s); and 9 days (placebo  $217 \pm 94$ , Dex  $644 \pm 71$ ,  $P < .05$ , Sham  $900 \pm 0$  s) (Fig. 1).

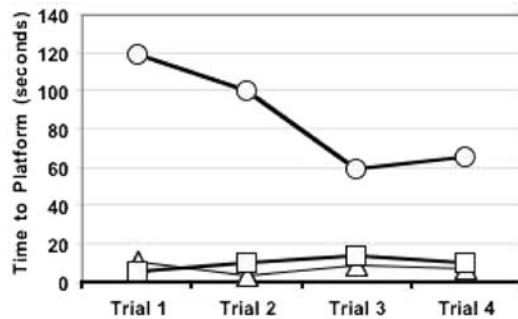
Performance in the water maze was improved in the dexamethasone groups compared to the placebo groups (Fig. 2 and Fig. 3). There was no difference between the Dex and sham groups. The swimming speed (length of the path divided by latency) was not significantly different between groups.

#### Serum C-tau and cerebral edema

Serum C-tau levels (ng/ml) was increased in infected animals in the group killed at 3 days; however, C-tau decreased below measurable levels in the group killed at 9 days. Dexamethasone significantly attenuated the C-tau

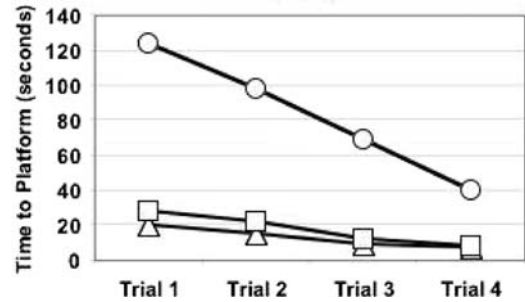


**Fig. 1** Sequential Rotorod trials group at 3 days and 9 days after randomization. Animals were pre-trained for 2 weeks. On the test day animals were given three trials of 15 min each walking at a speed of 25 revolutions per minute. Meningitis produces a decrease in this locomotor activity ( $P<.05$ ), dexamethasone attenuates this deficit ( $P<.05$ ).



**Fig. 2** Sequential water maze trials on groups at 3 days after randomization. Triangle ( $\Delta$ ) Sham, Circle ( $\lambda$ ) Meningitis, and Square ( $\theta$ ) dexamethasone-treated animals, time (mean in seconds) to locate a submerged platform on test day. Animals were pre-trained for 2 weeks, the criterion, 25 s, was calculated as the mean time $\pm$ 2SD for all animals from their final pretreatment test. On the test day animals were given four trials of 5 min. each to locate the submerged platform. Dexamethasone-treated animals were able to achieve criterion faster than the meningitis counterpart ( $P<.05$ ).

increase (placebo  $307\pm105$ , Dex  $21\pm9$ ;  $P<.05$ , Sham  $1.4\pm1.4$ ). Dexamethasone also reduced the brain water content of animals killed at 3 days (placebo  $78.76\pm0.19\%$ , Dex  $76.82\pm0.36\%$ ,  $P<0.001$ ). However, dexamethasone



**Fig. 3** Sequential water maze trials on groups at 9 days after randomization. All other details as in the previous figure

induced a weight loss at 3 days (placebo  $20\pm1\%$  vs Dex  $26\pm1\%$ ,  $P<.05$ ) that was not present in the group killed at 9 days (placebo  $19.2\pm1.8\%$  vs Dex  $16.5\pm2\%$ ,  $P=NS$ ).

## Protocol 2

The css were not significantly different between infected groups (Table 1).

## Caspase activation

Caspase 1 and 3 are activated in meningitis, with the highest values obtained 24 h after randomization. Caspase 1 differences between groups did not reach statistical significance. Dexamethasone produced a significant decrease in Caspase 3 activation that is evident 24 h and 72 h after its administration (Table 1).

## Discussion

Experience reveals that it is more difficult to effect neurobehavioral outcome than to produce interference with an injurious pathophysiologic process. Our most important finding is that dexamethasone attenuates the deterioration observed in neurobehavioral testing in meningitic animals and that this improvement is associated with a decrease in biological markers of brain injury.

**Table 1** Clinical scores and Biological markers protocol 2 (css clinical severity score, ND non-detectable).

	6 h			24 h			72 h		
	Sham	Placebo	Dex	Sham	Placebo	Dex	Sham	Placebo	Dex
Css	0	$0.6\pm0.1$	$0.75\pm0.2$	0	$0.75\pm0.1$	$0.7\pm0.1$	0	$0.7\pm0.1$	$0.7\pm0.2$
Caspase 1 (pmol/l $\pm$ SEM)	ND	$4.4\pm1.7$	$3.2\pm0.7$	ND	$7.3\pm1.6$	$3.9\pm1.8$	ND	$1.3\pm0.6$	$1.5\pm1.3$
Caspase 3 (pmol/ml $\pm$ SEM)	ND	$22\pm9.5$	$25.4\pm7.2$	ND	$19.6\pm0.8$	$4.0\pm1.2^{**}$	ND	$6.8\pm0.4$	$1.1\pm0.6^*$

\*  $P<.05$

\*\*  $P<.0001$

Two protocols were performed due to the fact that animals need to be killed to obtain biochemical markers and are very weak with signs of meningismus during the initial 48 h not permitting neurobehavioral testing.

The Rotorod test areas of the brain responsible for postural equilibrium and coordination, the results suggest that dexamethasone treated animals had fewer motor deficits. The water maze detects deficits of learning acquisition and memory, the results suggests that dexamethasone attenuates meningitis induced memory deficits [16]. We speculate that the improvement seen in the dexamethasone group is due to an enhancement in memory retention, because their previous training makes it problematic to attribute the differences observed to changes in learning acquisition. Interpretation of the water maze results is complicated by the fact that the water maze requires the animals to swim. Therefore, motor deficits secondary to brain injury may influence their performance. However, we do not feel this is a major factor as there was no difference in swim speed when the groups were compared.

We have established that the presence in serum of C-tau correlates with histological brain injury in this animal model [13]. An early increase in serum C-tau levels was observed in meningitic animals and the use of dexamethasone significantly decreased the rise in C-tau. We cannot rule out the possibility that a decrease in blood-brain barrier inflammation may alter the cerebral spinal

fluid/serum ratio of C-tau giving a falsely low serum level. The observed reduction in brain edema and improvement in neurobehavioral performance support the contention that dexamethasone minimizes CNS injury [17, 18].

Further, this study demonstrated that dexamethasone decreases Caspase 3 activation as early as 24 h after its administration. After an injury, mitochondrial and cell death receptor pathways converge to cause the activation of Caspase 3 that precedes DNA fragmentation and apoptosis [19]. We cannot conclude if the decrease in caspase activation is a direct effect of dexamethasone or an epiphenomenon to decrease inflammation, swelling, and improved perfusion. These results should also not be extrapolated to immature animals due to dexamethasone effects in transcription factor activating protein-1 that are age-dependent [20].

The current study demonstrates that dexamethasone produces and an improvement in serologic and neurobehavioral markers of neuronal injury in meningitis induced brain injury. Further, it offers direction for future studies with which to improve our understanding of the possible function of steroids in the treatment of brain injury.

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